



Biocatalytic alternative for bio-glycerol conversion with alkyl carbonates *via* a lipase-linked magnetic nano-particles assisted process

Madalina Tudorache, Alina Negoi, Loredana Protesescu, Vasile I. Parvulescu*

University of Bucharest, Department of Organic Chemistry, Biochemistry and Catalysis, Bd. Regina Elisabeta 4-12, Bucharest 030016, Romania

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ABSTRACT

Bio-glycerol (B-Gly) was converted to glycerol carbonate (GlyC) as an important added-value product using an advanced biocatalytic process involving the glycerol (Gly) carbonylation with dimethyl carbonate (DMC) assisted by a heterogeneous enzyme biocatalyst (lipase covalently attached on magnetic nano-particles surface). The biocatalytic process was set up optimizing the experimental parameters (e.g. molar ratio of the reagents, temperature, incubation time and catalyst content). In addition, the efficiency of DMC as carbonylation agent was evaluated against diethyl carbonate (DEC) and dibenzyl carbonate (DBC). B-Gly was tested as a raw material for GlyC synthesis in the set up biocatalytic process using commercial pure Gly (S-Gly) as reference. B-Gly was produced based on a conventional transesterification process applied to both crude and residual sun-flower oil. Crude sun-flower oil was the typical commercial one and residual sun-flower oil was that recovered from the cooking process. Comparable performances were obtained using B-Gly from residual oil and S-Gly (e.g. conversion of Gly of 36% and 45% for selectivity in GlyC of 90% and 92%, respectively), while the use of B-Gly from crude residual oil led to a Gly conversion of only 27% with selectivity in GlyC of 95%.

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1. Introduction

Actual economy and nowadays lifestyle rely on the fossil resources for most of the energy production. However, these natural sources are limited and their stock cannot be refilled in a short time. In addition, they are expensive and their exploitation has a negative impact on the environment [1]. These are the main arguments taken under consideration to launch the new world strategy with biomass as renewable carbon source. So far, the biomass composition used as feedstock imposed at least three general platforms, such as sugar, syngas and oil. The oil platform is well-established today, with biodiesel being one of the most popular commercial processes based on the oil as raw material. Biodiesel is a transesterification process of the triglyceride from oil matrix with a short chain alcohol (e.g. methanol and ethanol). A predominant by-product of biodiesel plant is glycerol (Gly) strategically named bio-glycerol (B-Gly) accredited as biomass product [2].

B-Gly is a key aspect of the biodiesel manufacture due to huge amount of Gly produced during the biodiesel process (e.g. 10 kg Gly for each 100 kg biodiesel produced) [3]. Unfortunately, this Gly possesses low value for industry because its impurity content (e.g. methanol, water, soap, salts, etc.) and also the purification alternative is not feasible for small-scale production [4]. The lack of

proper management for B-Gly entailed the decrease of the industry interest in biodiesel due to its high production costs. Another consequence is that B-Gly has become a potential environmental pollutant due to the large Gly amount stocked in the environment [5]. Thus, the rescue of the biodiesel market requests the implementation of new technologies involving B-Gly as raw material which certainly will lead to achieve greater economic, social and environmental quality for biodiesel industry [6].

While pure Gly found numerous industrial applications (e.g. in food, drug, cosmetic and tobacco industries) the utilization of B-Gly is highly limited due to its diverse composition. Thus, the use of B-Gly as an ingredient in the animal feed is among the few applications [7]. But even in this case, although the large amount of accessible B-Gly allows for using it like a low cost additive, an excess of Gly in the animal diet may affect the normal physiological metabolism and, overall, the animal health [8]. Nowadays, there are suspicions on the commercial quality of meat and eggs from these animals. Also, the effects of the Gly impurities on the animals health are currently under investigations [9]. Another economic strategy for exploitation of B-Gly is its transformation to added-value products *via* chemical or biochemical conversion [1,6]. B-Gly can be catalytically converted to hydrogen (H₂) or syngas (H₂/CO) based on the reforming processes [10–13]. Fermentative metabolism of B-Gly is of special interest for 1,3-propanediol production [14]. Also, there is a wide variety of chemical products which can be prepared using B-Gly as raw material (e.g. propylene glycol, succinic acid, polyesters, lactic acid, polyglycerols, among others) [3,15–18].

* Corresponding author. Tel.: +40 21 4100241; fax: +40 21 4100241.

E-mail address: vasile.parvulescu@unibuc.ro (V.I. Parvulescu).

Glycerol carbonate (4-hydroxymethyl-1,3-dioxolan-2-one) (GlyC) is one of the most relevant examples of added-value products derived from B-Gly. GlyC is a relative new material with large potential in the chemical industry as “green” solvent, component in coatings, paints, and detergents and also as a source of polycarbonates and polyurethanes [19,20]. It has convenient properties (e.g. low toxicity, good biodegradability and high boiling point) to be used in the industrial processes characterized by green treasures. As glycerol-derivatives, GlyC can be prepared based on catalytic transesterification of linear (e.g. dimethyl carbonate and diethyl carbonate) or cyclic (e.g. ethylene carbonate and propylene carbonate) organo-carbonates with Gly in the presence of metal-catalysts (ZnCl_2), basic oxides (CaO), hydrotalcites or ionic liquids [21–24]. Oxidative carbonylation or catalytic carbonation of Gly are other viable routes for GlyC synthesis [25–28].

In this paper we report a biocatalytic solvent-free route for the GlyC synthesis via transesterification in an excess of DMC. The biocatalyst has a heterogeneous design with the lipase enzyme from *Aspergillus niger* (A. niger) source covalently immobilized onto a magnetic nano-particle surface. The selection of the lipase enzyme from A. niger source is the result of an enzyme screening reported elsewhere [29]. The biocatalyst preparation and the characterization of the enzyme covalently attached to the functionalized surface of magnetic nano-particles have also been described in a previous paper [30].

In this study we report an advanced heterogeneous biocatalytic process that was set up optimizing the most influenced experimental parameters (molar ratios of the reagents, temperature, incubation time and catalyst content). In addition, different carbonylation agents (DEC and DBC) instead of DMC were tested as well. One of the innovative aspects of this paper is the use of B-Gly as substrate for biocatalytic synthesis of GlyC. The performances of the method are comparatively presented for real and standard samples, i.e. unrefined B-Gly and S-Gly.

2. Experimental

2.1. Chemicals and solutions

The substrates used in this study: glycerol (S-Gly), DMC, DEC, and DBC were purchased from Sigma–Aldrich (USA). Lipase enzyme from *Aspergillus niger* source was also obtained from Sigma–Aldrich (USA). The enzyme was immobilized on the surface of magnetic nano-particles with 50 nm external diameter and covering layer of PEA (polyethylenamine) with $-\text{NH}_2$ functional group used for enzyme immobilization (Chemicell, Rostock, Germany). 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were used as immobilization reagents. A solution of 0.1 M 2-(N-morpholino)ethanesulfonic acid (MES), pH 4.7/7.4 was used as a coupling buffer for the enzyme attachment. Phosphate buffer saline (PBS) of 10 mM concentration (8 g NaCl, 0.2 g KCl, 1.43 g $\text{Na}_2\text{HPO}_4 \times 2\text{H}_2\text{O}$ and 0.34 g KH_2PO_4 in 0.1 L distilled water) at pH 7.4 containing 0.1% BSA and 0.05% sodium azide was used for blocking the active sites of the solid surface after the enzyme immobilization. All the reagents used for the biocatalyst preparation were purchased from Sigma–Aldrich (USA). Derivatization reagents (BSTFA: TMCS = 99:1 and pyridine) were purchased from Macherey–Nagel Corp. (Duren, Germany) and Fluka (Switzerland). The organic solvents used in all the experiments were of analytic purity.

2.2. Biocatalyst preparation

Lipase enzyme from *Aspergillus niger* source was covalently immobilized on the magnetic nano-particles according to the

approach already reported in the literature [30]. 0.1 mL suspension of magnetic beads (nano-particles of 50 nm external diameter and shell structure with Fe_3O_4 magnetic core and PEA external coverage) were placed in an Eppendorf vial where the beads were separated from the solution using a permanent magnet. The particles were washed with 0.1 M MES by separation and re-suspension in the liquid phase. Then, a solution containing 10 mg/mL EDC and 43 $\mu\text{g/mL}$ of lipase dissolved in 0.1 M MES (pH 4.7) were added in the Eppendorf vial containing the magnetic support pre-treated with MES. The suspension was stirred gently for 2 h. The resulted biocatalyst lipase-beads were washed with a PBS solution (0.1 M, pH 7.4) for 3 times and re-suspended in the blocking solution (0.1% BSA and 0.05% sodium azide in 0.1 M PBS) to remove the enzyme excess and to avoid the unspecific binding on the particle surface.

2.3. Biocatalytic synthesis of GlyC

Biocatalytic synthesis of GlyC has been set up converting the Gly substrate using the carbonylation reagent of DMC into GlyC product under solvent-free conditions. The process was catalyzed by previously prepared lipase-beads. Given amounts of Gly and DMC at different Gly: DMC molar ratios (e.g. 1:1, 1:2, 1:4, 1:5, 1:10, 1:16, 1:20, 1:25 and 1:50) were mixed together with the lipase-beads catalyst (1, 2.5, 5, 7.5, 10 and 20%, w/w) in a 1.5 mL reaction vial (Eppendorf tube). The mixtures were incubated for maximum 24 h at temperatures in the range 25–80 °C under vigorous stirring using a thermostatic shaker. After the reaction was completed, the biocatalyst was recovered from the reaction mixture using a magnetic field. A permanent magnet was placed closed to the wall of the reaction vessel. In this way, the biocatalyst was separated from the reaction mixture. The liquid phase was evaporated at 50 °C under vacuum in order to eliminate the excess of DMC and the produced methanol. Dried reaction products (GlyC and secondary products) were analyzed using gas chromatography (GC) coupled to flame ionization (FID) or mass spectrometry (MS) detector.

2.4. Determination of the synthesis products

The analysis of Gly and reaction products required silylation before the injection in the chromatographic column, in order to obtain proper peak shapes, and also a low detection limit [31]. For this purpose, 100 μL silylation agent (BSTFA: TMCS = 99:1) were added to the reaction samples (after the evaporation step), and then the resulted mixture was diluted with 100 μL pyridine. Derivatization process has been performed under gently agitation at 60 °C, for 30 min. Before analysis, 100 μL of *n*-heptane were added as internal standard.

The composition of derivatized samples (1 μL) was determined using GC–MS based on Trace GC 2000 system incorporating a MS detector (Thermo Electron Scientific Corporation, USA) and TR-WAX capillary column. The injection chamber was set up at 200 °C and the temperature in the detector cell was 270 °C. The temperature in the oven was kept constant at 50 °C for 1 min and then it was increased at 250 °C with a 10 °C/min rate. Finally, the oven temperature was maintained at 250 °C for 3 min.

Also, the same samples were analyzed with a GC-FID (Schimadzu GC-2014, Thermo Electron Scientific Corporation, USA) chromatograph equipped with TR-WAX and TR1MS capillary columns using hydrogen as a carrier gas (1.0 mL/min). The detector and injector were set up at the temperature of 250 °C. The oven program was similar with that used for the GC-FID analysis. The chromatograms were used for the calculation of the Gly conversion (C_{Gly}) and GlyC selectivity (S_{GlyC}) as following:

$C_{\text{Gly}} (\%) = (\text{sum of peak areas of the reaction products} / \text{sum of all peak areas of the chromatogram}) \times 100$

$$S_{\text{GlyC}} (\%) = (\text{GlyC peak area} / \text{sum of peak areas of the reaction products}) \times 100$$

3. Results and discussions

3.1. Influence of the reagents ratio on the GlyC synthesis

As mentioned, the GlyC synthesis was performed based on a carboxylation process of Gly with DMC that was catalyzed by the lipase-linked magnetic nano-particle composite. According to the literature [32], the reaction mechanism followed the chemical route presented in Scheme 1. Gly and DMC interact leading to an unstable intermediate (3) and methanol. Further, this intermediate follows an intramolecular cyclisation removing one methanol molecule and generating GlyC (4). Secondary products of this process can be glycerol dicarbonate (GlyDC) (5) and diglycerol tricarbonate (DGlyTC) (6) [29]. Considering the theoretical route of Gly carbonylation (Scheme 1), the reaction stoichiometry requires 1 mol of DMC for every mol of Gly (equimolar ratio Gly:DMC). However, the reaction mechanism is differently influenced by the reagents ratio (Gly:DMC) for inorganic and enzymatic catalyzed reaction [29,32]. Base inorganic catalysts (e.g. K_2CO_3) favor the formation of GlyC using only 3-folds of DMC excess, while secondary products (e.g. GlyDC and DGlyTC) resulted for a large excess of DMC (10-folds) and high temperatures (e.g. 95°C) [32]. Via enzymatic biocatalysis, GlyC is produced for 2-fold of DMC excess in organic solvent (THF) or 10-fold DMC excess in solvent-free conditions [29,33,34].

In this study, the mechanism of the interaction between Gly and DMC was checked using the heterogeneized biocatalyst (i.e. lipase from *Aspergillus niger* immobilized on magnetic nano-particle). The experimental data are presented in Fig. 1. A low molar Gly:DMC ratio (Gly:DMC = 1:50) led to an insignificant Gly conversion (2.2%). However, the Gly conversion was enhanced when the Gly:DMC ratio acquired higher values. Thus, the Gly conversion varied in the range of 7.2–45% when the Gly:DMC molar ratio varied from 1:25 to 1:10 (Fig. 1). Then, the Gly conversion experienced a downward trend for a DMC excess lower than 5-folds (Fig. 1). The selectivity in GlyC was negatively influenced only by high Gly contents (e.g. Gly:DMC molar ratios of 1:2 and 1:1) where the DGlyTC production was favored (Fig. 1). The highest Gly conversion was achieved for a 10-fold of DMC excess. This ratio also provided solvent-free conditions of the system.

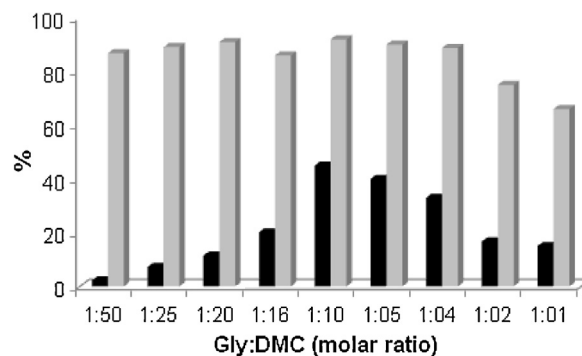
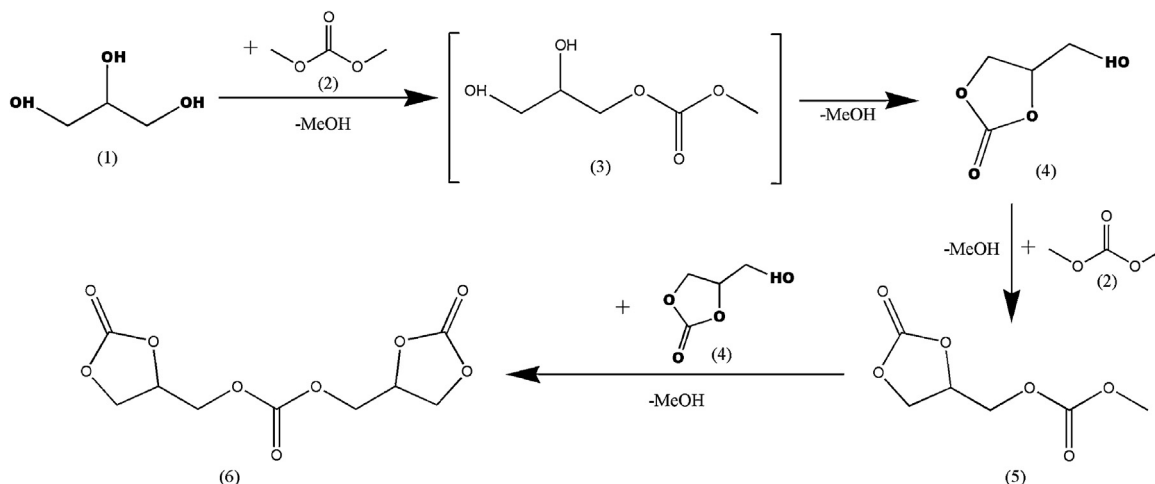


Fig. 1. The influence of the reagents ratio (Gly:DMC) on GlyC synthesis. Conditions: 5% (w/w) biocatalyst, 60°C and 6 h incubation time (Gly conversion – black square, selectivity in GlyC – gray square).

Variation of the Gly conversion as a function of the Gly:DMC molar ratio is specific to lipases [35]. In contrast to other enzymes, lipase does not recognize the substrate under its critical concentration (i.e. minimum substrate concentration visible for enzyme) and displays an increasing activity for increased substrate concentrations [35]. Thus, higher Gly concentrations as 1:50 Gly:DMC molar ratios led to the product. The maximum of the Gly conversions was achieved for concentrations corresponding to a Gly:DMC molar ratio of 1:10 (Fig. 1). However, the enhancement of the lipase activity is limited by the enzyme saturation with the substrate (saturation concentration of the substrate) [35]. In our specific case it corresponded to a Gly:DMC molar ratio of 1:10 (Fig. 1). Experiments with Gly:DMC molar ratios in the range 1:10 to 1:1 confirmed this fact. Theoretically, the lipase activity has to be independent of the substrate concentration for higher values than the saturation one. However these results show that when the lipase substrate is glycerol the theory is infirmed. High Gly concentrations enable the agglomeration of glycerol molecules around the lipase thus influencing its catalytic performance [22].

3.2. Influence of the biocatalyst loading

Black experiments were carried out considering both the reaction of glycerol with DMC in the absence of the enzyme and in the presence of the magnetic particles (the biocatalyst support).



Scheme 1. Schematic representation of GlyC synthesis. (1) glycerol (Gly); (2) dimethyl carbonate (DMC); (3) unstable intermediate; (4) glycerol carbonate (GlyC); (5) glycerol dicarbonate (GlyDC) and diglycerol tricarbonate (DGlyTC).

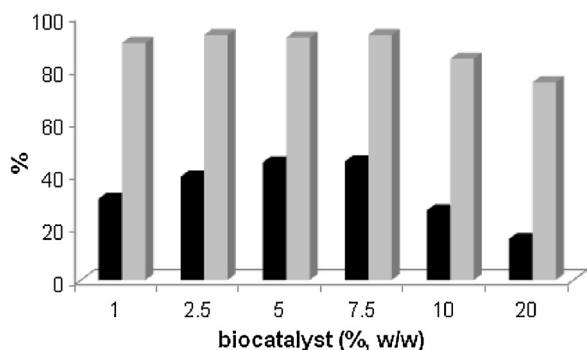


Fig. 2. The influence of the catalyst content of the GlyC synthesis. Conditions: Gly:acylation reagent molar ratio of 1:10, 60 °C and 6 h incubation time (Gly conversion – black square, selectivity in GlyC – gray square).

Glycerol was not converted to GlyC neither in the absence of the biocatalyst nor in the presence of the free support.

Fig. 2 illustrates the variation of Gly conversion and selectivity in GlyC as a function of the biocatalyst loading in the reaction mixture. The effective content in the biocatalyst was calculated as a ratio between the immobilized enzyme amount and the total mass of the reaction mixture (% w/w). The biocatalyst loading varied in the range of 1–20% (w/w). No linear dependence between the conversion and the catalyst loading has been determined. As expected, in the range of the low loadings, the Gly transformation was enhanced in the presence of larger loadings of biocatalyst. Therefore, the conversion of Gly increased from 31% corresponding to a loading of 1% (w/w) biocatalyst to 45% for a loading of 5% (w/w) biocatalyst, while a further increase of the loading to 7.5% (w/w) biocatalyst exhibited no effect. Further, the increase of biocatalyst amount led to a decrease of the Gly conversion (Fig. 2). A similar behavior has been, however, determined when the reaction has been carried out under homogeneous conditions with the same lipase from *Aspergillus niger* biocatalyst [29]. This behavior is assigned to the intermolecular interaction of lipase enzyme molecules, which for certain enzyme loading is leading to a blockage of the catalytic sites.

Based on the experimental data, we assume that 5% (w/w) enzyme loading correspond to the covering the support surface. The increase of the enzyme amount led to larger enzyme–enzyme interactions instead of enzyme–support interactions. For such loadings enzyme molecules will block reciprocally with visible effect on the catalytic results (see 7.5%, w/w). Such a behavior is specific for protein molecules, and for lipase as well [36].

The selectivity in GlyC was not substantially influenced by the variation of biocatalyst content and its value was roughly constant around 90%.

Based on these experiments a biocatalyst loading of 5% (w/w) was selected for further experiments. It corresponded to a Gly

conversion of 45% and a selectivity in GlyC of 92%. It is thus important to notice that under the homogeneous conditions comparable results were obtained for a loading of 12% (w/w) *Aspergillus niger* [29] while with the heterogeneous catalyst the same performances were obtained with a loading that was twice smaller (5%, w/w). These data demonstrate that the immobilization of lipase provides a more efficient biocatalyst. Calculated TON values corresponded to 20.4×10^5 for the immobilized enzyme and 4.64×10^5 for the free lipase.

3.3. The effect of the temperature and incubation time on the GlyC synthesis

Fig. 3 shows data collected for the different temperatures of reaction (Fig. 3A) and incubation times (Fig. 3B). The temperature varied in the range 25–80 °C (Fig. 3A). Gly conversion firstly increased gradually until the temperature reached the value of 60 °C and then exhibited a slight decrease. Interestingly, the selectivity in GlyC remained unchanged even when the temperature was raised up to 80 °C (Fig. 3A). The increase of the conversion for temperatures lower than 60 °C is assigned to a progressive thermal energy provided to the biocatalytic system without affecting the biocatalyst configuration. The regressive profile of the Gly conversion is a direct consequence of the lipase denaturation for temperature higher than 60 °C [37,38]. A similar effect of lipase denaturation was also observed under homogeneous conditions [29]. However, while under the homogeneous conditions the Gly conversion was diminished with 20% [29], under heterogeneous conditions the depletion of Gly conversion was of only 5% when the temperature was raised from 60 to 70 °C.

It appears that the heterogeneity of the catalyst stabilizes the enzyme leading to a more robust system.

GlyC conversion was also monitored for different incubation times as it results from Fig. 3B. Equilibrium conditions were almost established after 6 h (e.g. 45% Gly conversion and 92% selectivity in GlyC). The extension of the incubation time until 24 h did not generate any visible benefit for the Gly conversion (only 45.3% Gly conversion). Furthermore, the selectivity in GlyC decreased favoring the formation of DGlyTC (e.g. a decrease in the selectivity of GlyC at 60% and an increase in selectivity of DGlyTC at 37%).

3.4. The effect of Gly carbonylation with different reagents

GlyC synthesis has been carried out using different acyl reagents for Gly carbonylation (e.g. DEC and DBC). As reference for these experimental results were the results obtained with DMC as carbonylation reagent (Table 1). Using DEC Gly was insignificantly converted (3.4% conversion of Gly), even if the dominant product was GlyC (>99% selectivity in GlyC) (Table 1). Similar performances of Gly conversion were achieved using DBC (Gly conversion <0.1%).

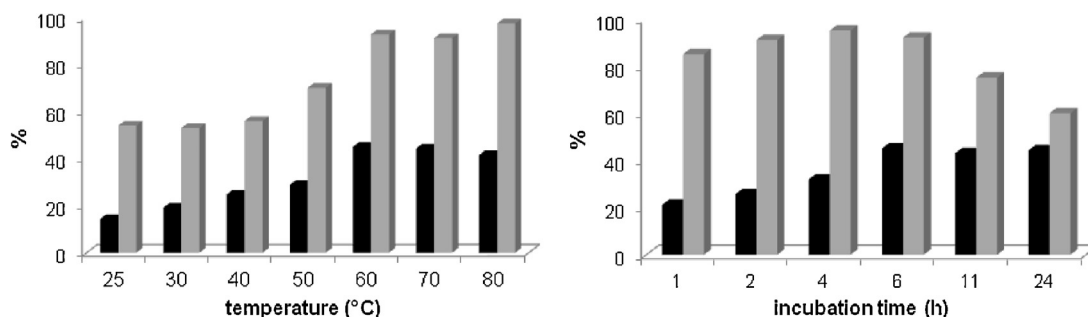


Fig. 3. Effects of temperature (A) and incubation time (B) on the GlyC synthesis. Conditions: Gly:acylation reagent molar ratio of 1:10, 5% (w/w) biocatalyst (Gly conversion – black square, selectivity in GlyC – gray square).

Table 1

Gly conversion for different routes of Gly carbonylation using DMC, DEC and DBC as acyl donor. Conditions: Gly:acylation reagent molar ratio of 1:10, 5% (w/w) biocatalyst, 60 °C and 6 h incubation time.

reagent	C _{Gly} (%)	S _{GlyC} (%)	S _{DGlyTC} (%)
DMC	45	92	7
DEC	3.4	>99	–
DBC ^a	<0.1	–	–

DMC – dimethyl carbonate; DEC – diethyl carbonate; DBC – dibenzyl carbonate; C_{Gly} – conversion of Gly; S_{GlyC} – selectivity in GlyC; S_{DGlyTC} – selectivity in DGlyTC.

^a The reaction was performed in THF organic solvent.

However, DBC was degraded under the experimental conditions by hydrolysis leading to benzyl alcohol. This process was confirmed by GC–MS analysis of the final reaction mixture. Hydrolysis of DBC even in the presence of traces of water was also reported in the literature previously [39].

3.5. GlyC synthesis using unrefined B-Gly

Optimal experimental conditions for the synthesis of GlyC using standard Gly corresponded to a Gly:DMC molar ratio of 1:10, 5% (w/w) loading of biocatalyst, 60 °C and 6 h incubation time. As it was mentioned above working under these conditions led to a conversion of 45% Gly and selectivity in GlyC of 92%. The biocatalyst was re-used for fifteen reaction cycles without significant variations of the biocatalyst performances (*i.e.* variation of TON value of the biocatalyst in the range of 0–5%). B-Gly samples produced as the mean by-product of biodiesel process developed for crude and residual sun-flower oil were used as well. After the separation from the biodiesel mixture, B-Gly was directly used for GlyC synthesis under the above conditions optimized. The results are presented in Table 2. Gly conversion around 27% and selectivity of 95% in GlyC were obtained for B-Gly separated from crude oil. Under similar conditions, using residual oil as substrate the Gly conversion of 35% with the selectivity in GlyC of 90% was achieved. Surprisingly, the B-Gly samples entailed different performance of GlyC synthesis process, even the biomass pattern was similar (sun-flower). However, the feedstock oils were differently treated before biodiesel process, which changed the matrix of the B-Gly. Crude sun-flower oil was the typical commercial one, while residual sun-flower oil was that recovered from the cooking process. In other words, crude oil was the oil sample provided from supermarket without any pre-treatment step, while residual oil was the oil used before in the cooking process. Consequently, the impurities content of the samples were different from 29.4% of methanol and 28.7% of water for crude oil to 6.5% of methanol and 29.8% of water for residual oil (Table 2). Therefore, higher Gly content was found for residual oil (around 64% Gly) compared to crude oil (around 42% Gly). A direct consequence of the B-Gly composition was reflected on the system performance for GlyC synthesis, *e.g.* better performance on GlyC synthesis for residual oil case (Table 2). On the other side,

Table 2

GlyC synthesis from B-Gly. Conditions: 25 mg B-Gly, 500 µL DMC, 5% (w/w) biocatalyst, 60 °C and 6 h incubation time.

Feedstock pattern for B-Gly	C _{Gly} (%)	S _{GlyC} (%)	RC _{Gly} (%)	Impurities content of B-Gly	
				MeOH (%)	H ₂ O (%)
Crude sun-flower oil	26.8	95	60	29.4	28.7
Residual sun-flower oil	35.3	90	78	6.5	29.8

C_{Gly} – conversion of Gly; RC_{Gly} – relative conversion of Gly related to conversion of standard Gly (45%) on GlyC synthesis.

methanol and also water impurities can strongly affect the biocatalytic activity of lipase and also the equilibrium of the reaction. This is also important aspect that must be considered. Higher methanol content of crude oil (29.4% of methanol) is another reasonable reason for only 59.7% recovery of the Gly conversion compared to 78% recovery of Gly conversion for residual oil which contained almost 6.5% methanol (Table 2).

4. Conclusions

An advanced biocatalytic process for GlyC synthesis based on carbonylation of Gly with DMC has been developed using heterogeneous lipase biocatalyst with the enzyme attached on the magnetic nano-particles surface. The biochemical route for GlyC synthesis shows “green” characteristics because the synthesis is performed under solvent-free conditions with DMC playing the role of “organic solvent” besides the carbonylation reagent value. Also, the developed biocatalytic system is environment friendly (*i.e.* the reagents and products of the biocatalytic process are not toxic) and the reaction is developed under mild experimental conditions (*e.g.* 60 °C temperature and atmospheric pressure). The use of heterogeneous biocatalyst allows the synthesis of GlyC with better efficiency compared to the case of homogeneous biocatalyst (*i.e.* 20.4 × 10⁵ and 4.64 × 10⁵ TON of immobilized and free lipase, respectively). A low enzyme loading is required for the biocatalyst preparation (500 µL of 43 µg/mL lipase solution), and low biocatalyst loading in the reaction mixture (*e.g.* 5%, w/w biocatalyst).

The investigated immobilized enzyme showed a good biocatalyst recyclability and stability. The catalytic capacity was preserved for fifteen cycles without significant modifications [30].

An important aspect of this study is that the developed system shows high robustness as far as the biocatalytic performances were conserved for the B-Gly substrate. Noteworthy, this property has been demonstrated working with B-Gly from different sources (*e.g.* crude and residual sun-flower oil). This is clear evidence that the lipase-beads biocatalyst preserves the catalytic properties even under the presence of the B-Gly impurities (methanol and water).

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